

Figure S1. Growth curves of CF longitudinal isolates. The indicated strains were grown for 16 hours in LB, then diluted in DTSB to an absorbance of 0.08 at 600 nm. The absorbance of the cultures at 600 nm was taken every 30 minutes in a Bioscreen C instrument. Cultures were grown with or without 100 μ M FeCl₃ supplementation as indicated. Error bars represent the standard deviation of three independent experimental cultures.

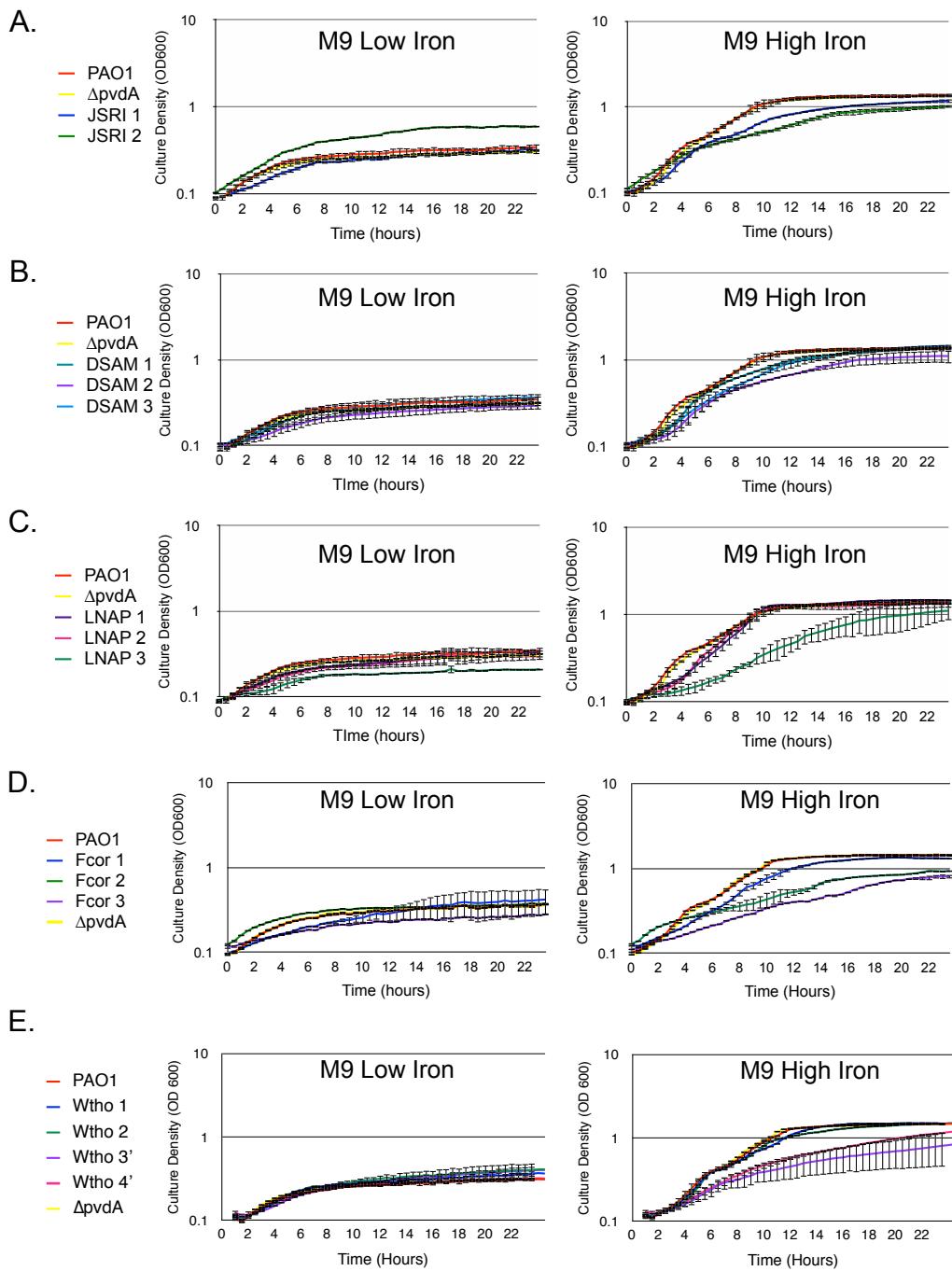


Figure S2. Growth curves of CF longitudinal isolates. The indicated strains were grown for 16 hours in LB, then 1:25 in M9, grown for an additional 4 hours, and diluted in fresh M9 media to absorbance of 0.08. The absorbance of the cultures at 600 nm was taken every 30 minutes in a Bioscreen C instrument. Strains were grown with or without 100 μ M FeCl₃ supplementation as indicated. Error bars represent the standard deviation of three independent experimental cultures.

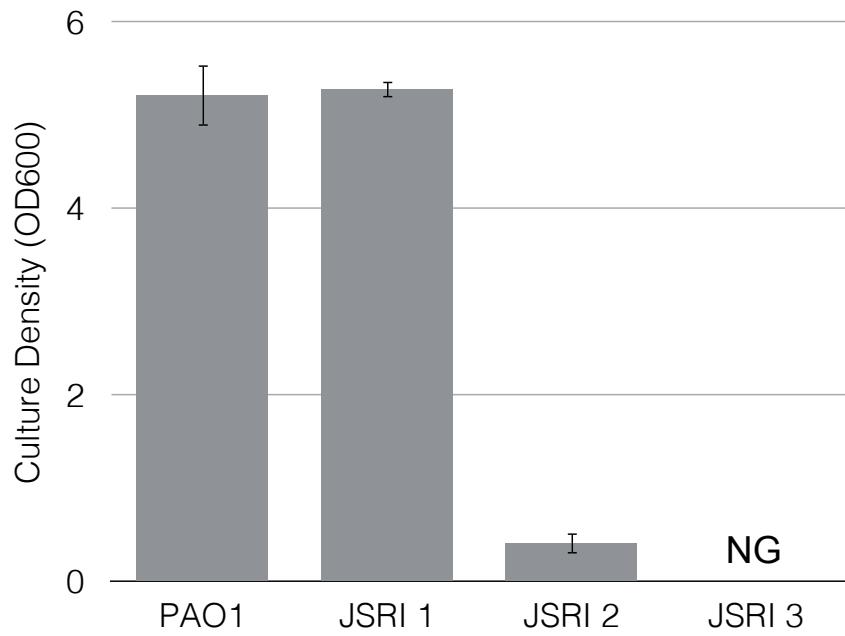


Figure S3. Growth of JSRI-3 is defective LB broth. The indicated strains were grown in LB broth for 16 hours, and the optical density of the culture was determined at 600 nm. Error bars represent the standard deviation of three independent experiments. NG – no growth detected.

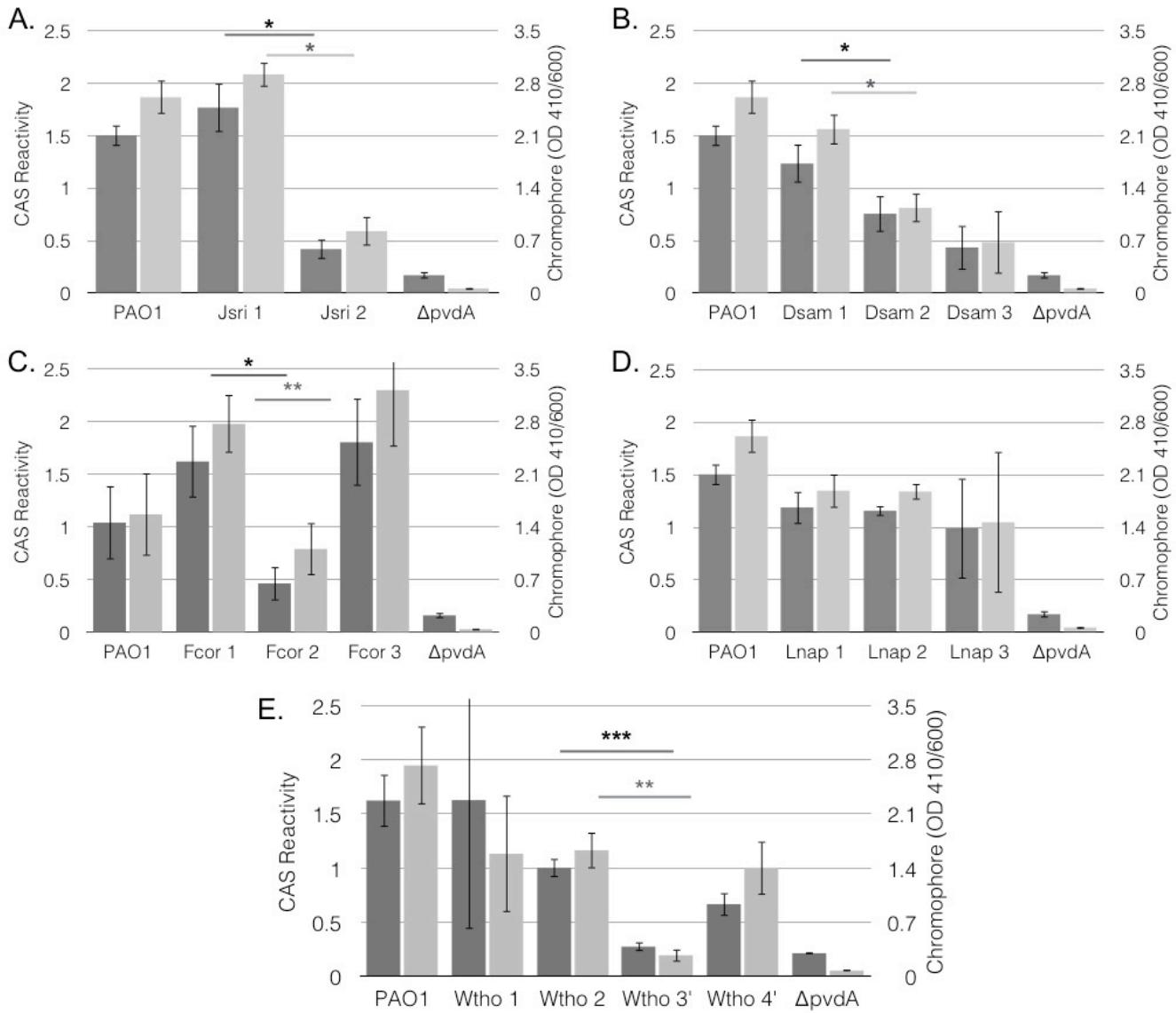


Figure S4. Pyoverdine and siderophore production in M9 media. JSRI, DSAM, FCOR, LNAP, and WTHO strains were grown for 16 hours in LB, diluted 1:25 in M9 and grown for 4 hours, then diluted into fresh M9 media to an absorbance of 0.08 and grown for an additional 8 hours. Cells were then harvested and supernatants analyzed for CAS reactivity (dark grey bars) and pyoverdine chromophore production (light grey bars) as described in materials and methods. Error bars show the standard deviations of three independent experiments. Asterisks indicate a significant change (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$) as determined by a two-tailed Student's t test.

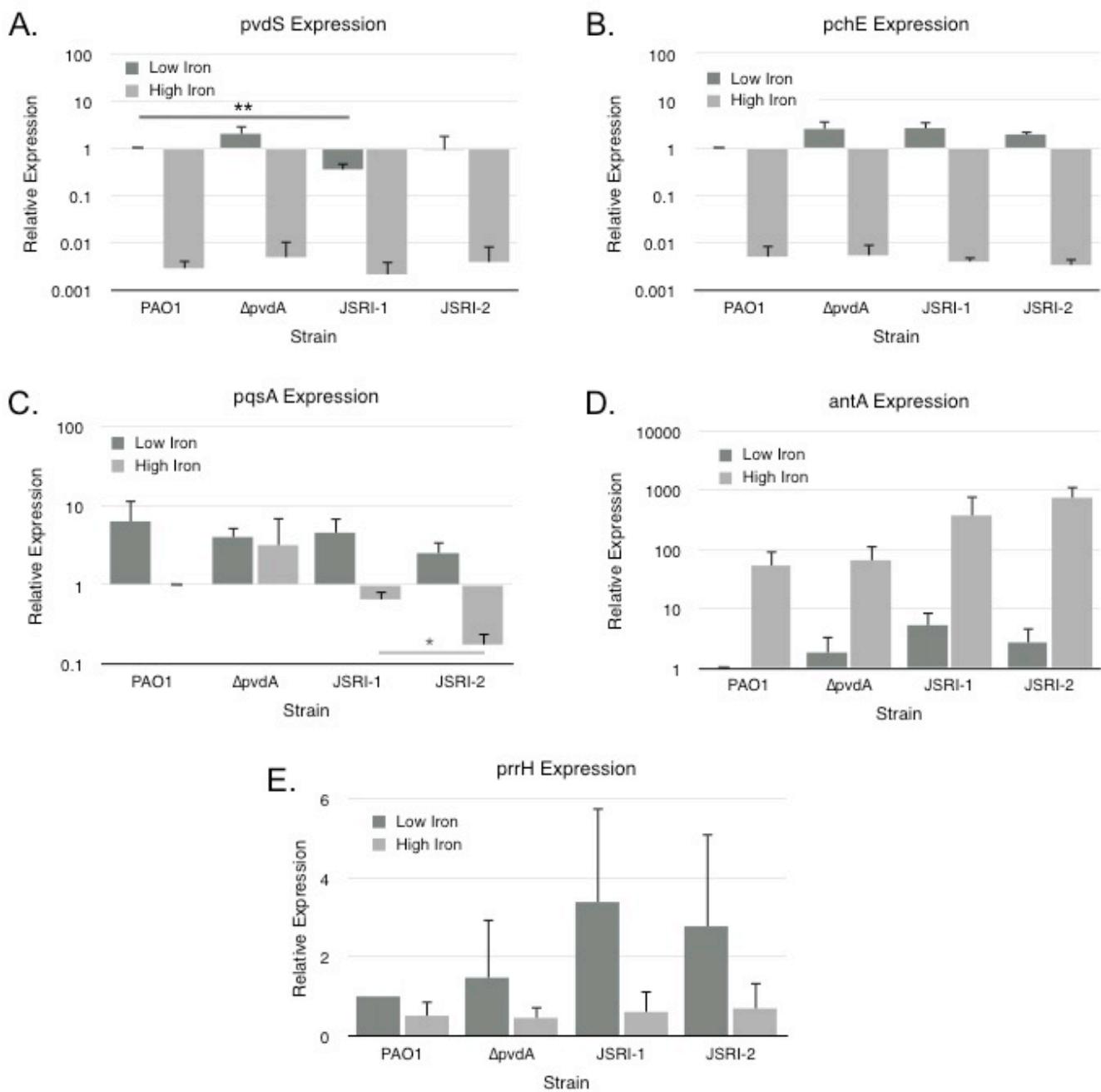
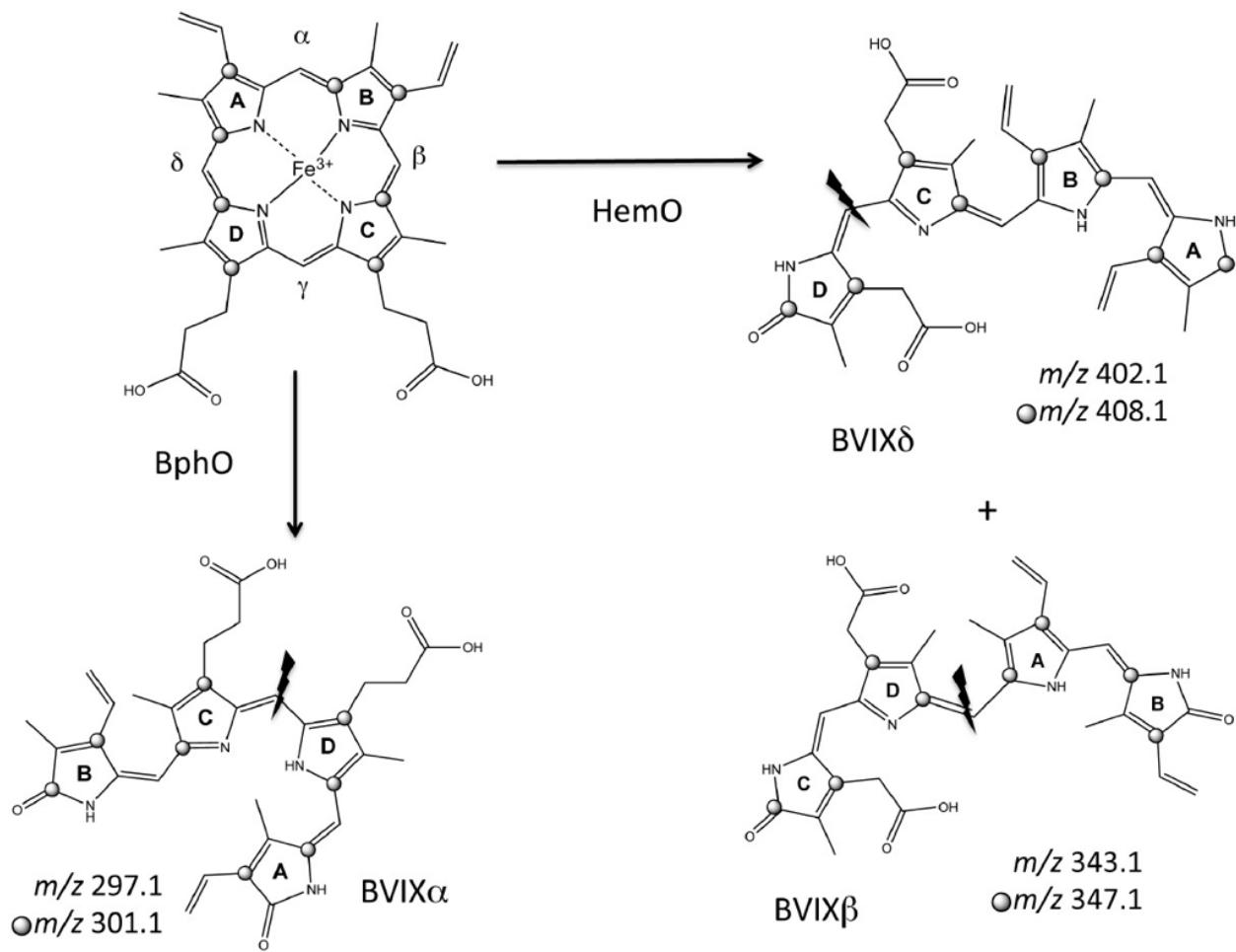


Figure S5. Relative expression of iron-regulated genes in the JSRI strain. RNA was isolated from the indicated strains grown in DTSB with or without 100 μ M FeCl₃ supplementation, and real time PCR was performed to analyze the abundance of the indicated transcripts as described in the materials and methods. Relative expression was determined using $\Delta\Delta Ct$, comparing the expression value of each sample to the PAO1 low iron sample (set at 1). Error bars indicate the standard deviation of three independent experiments. Asterisks indicate a *p* value of less than 0.05 (*) or 0.005 (**) as determined by a two-tailed Student's *t* test.



Scheme 1. MS/MS Fragmentation patterns of the ¹³C-labeled and unlabeled BVIX isomers. ¹³C-labeling pattern marked by grey circles leads to an increase of eight mass units for heme and the respective fragment ions of the respective BVIX isomers as shown.

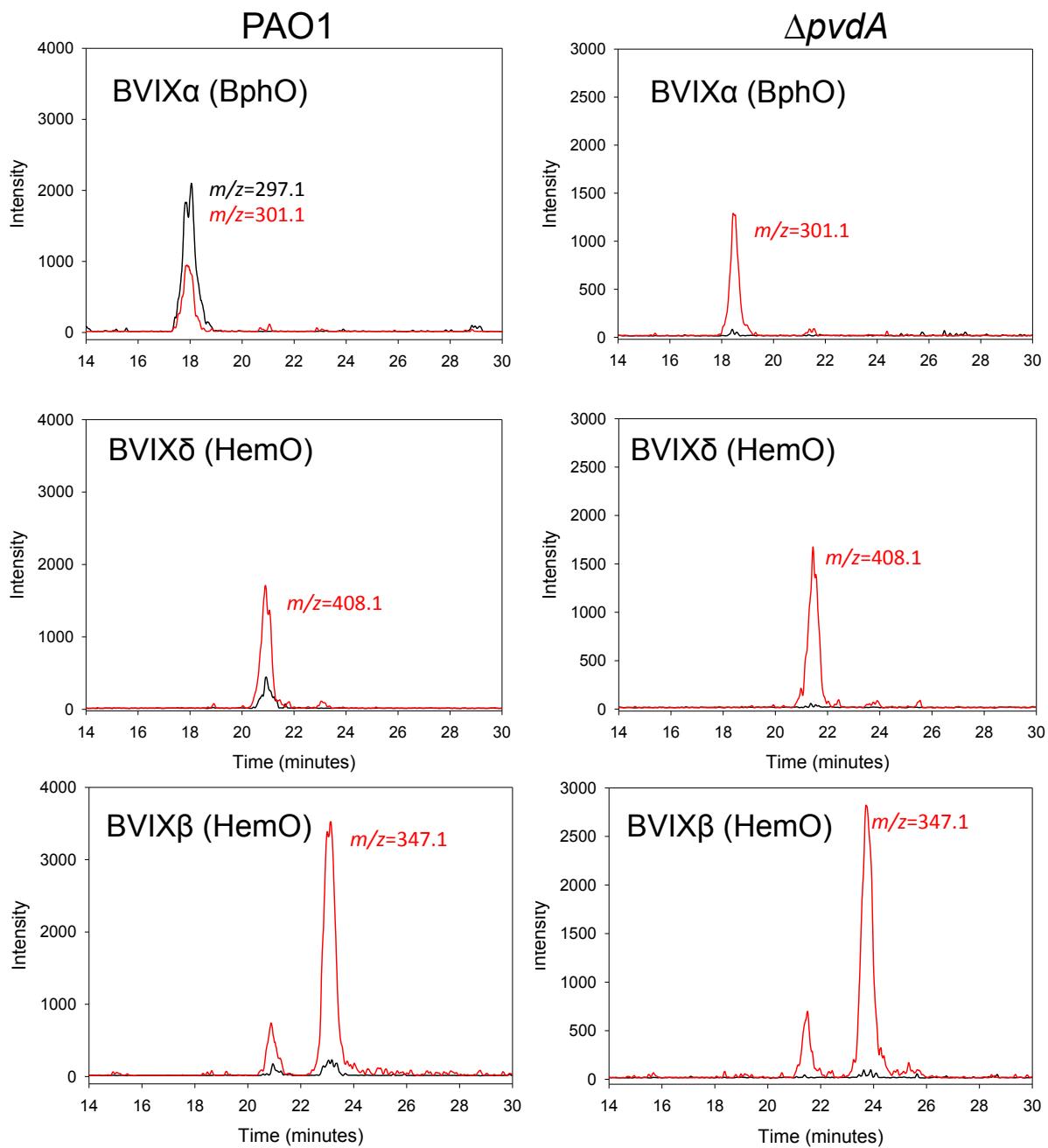


Figure S6. LC-MS/MS BVIX isomer fragmentation patterns for PAO1 and $\Delta pvdA$ strains supplemented with 5 μM ^{13}C -heme. MS/MS fragmentation of ^{13}C (red line) and ^{12}C (black) BVIX. LC-MS/MS was performed as described in the Materials and Methods with multiple reaction monitoring.

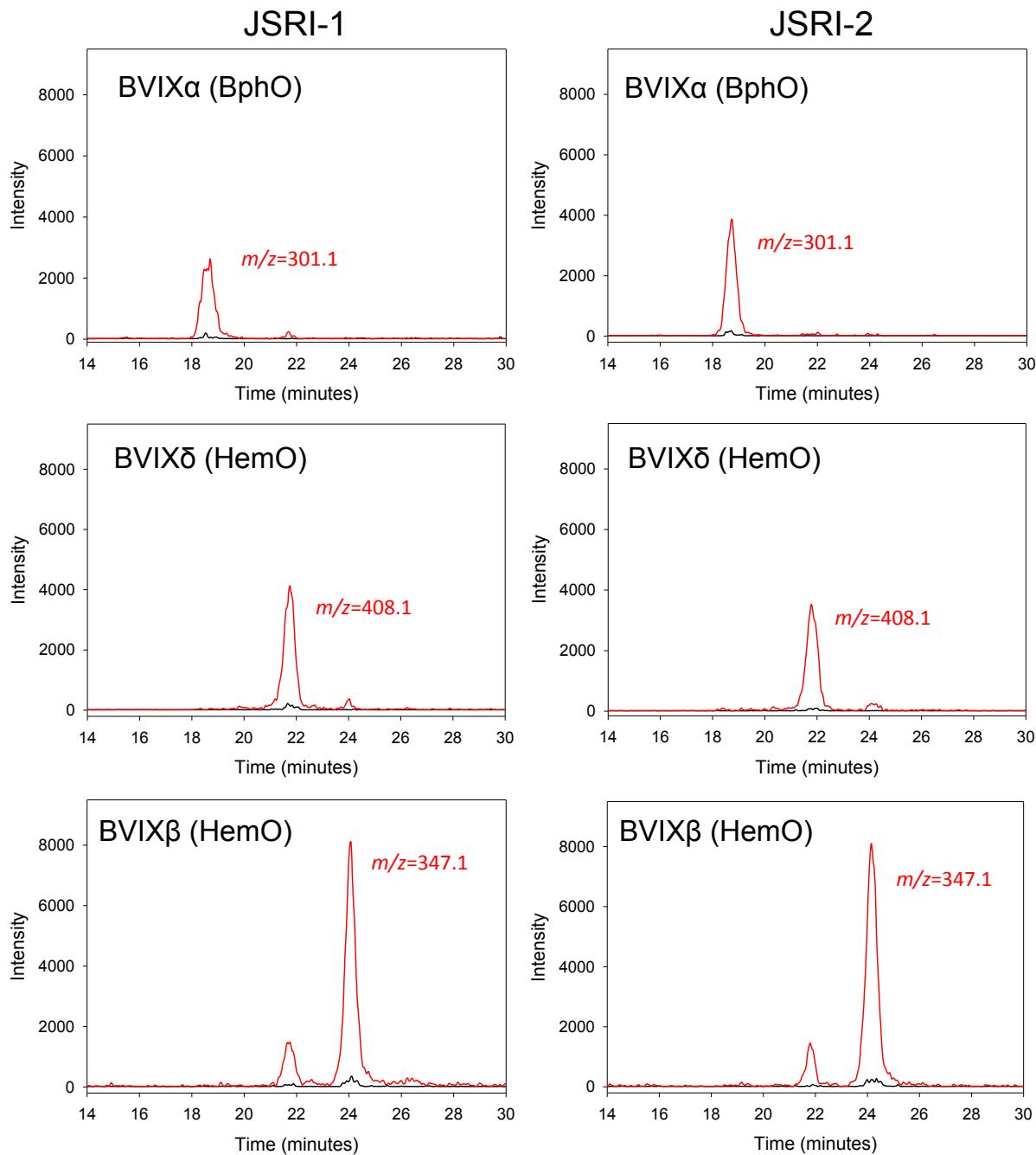


Figure S7. LC-MS/MS BVIX isomer fragmentation patterns for JSRI-1 and JSRI-2 isolates supplemented with 5 μM ^{13}C -heme. MS/MS fragmentation of ^{13}C (red line) and ^{12}C (black) BVIX. LC-MS/MS was performed as described in the Materials and Methods with multiple reaction monitoring.

Table S1. Bacterial strains and plasmids used in this study.

Strain/Plasmid	Description	Source/Reference
<i>Strains</i>		
SM10 λpir	<i>E. coli</i> strain used for conjugation: <i>pirR6K</i>	Taylor, et al. ¹
PAO1	Wild type <i>P. aeruginosa</i> strain used for mutational analysis in this and previous studies. Obtained from Dr. Mike Vasil.	Holloway ²
PAK	Wild type <i>P. aeruginosa</i> strain obtained from Dr. Mike Vasil.	M. Vasil
PA103	Wild type <i>P. aeruginosa</i> strain obtained from Dr. Mike Vasil.	M. Vasil
PA14 - MV	Wild type <i>P. aeruginosa</i> strain obtained from Dr. Mike Vasil.	M. Vasil
PA14 - DN	Wild type <i>P. aeruginosa</i> strain obtained from Dr. Dianne Newman.	D. Newman
Δ <i>pvdA</i>	Deletion of <i>pvdA</i> gene generated previously in PAO1	Ochsner, et al. ³
Δ <i>pvdD</i>	Deletion of <i>pvdD</i> gene generated previously in PAO1	Ochsner, et al. ³
Δ <i>pchEF</i>	Deletion of <i>pchEF</i> genes generated previously in PAO1	Banin, et al. ⁴
Δ <i>pvdDΔpchEF</i>	Deletion of <i>pchEF</i> and <i>pvdD</i> genes generated previously in PAO1	M. Vasil
Δ <i>pvdS</i>	Deletion of <i>pvdS</i> gene generated previously in PAO1	Ochsner, et al. ³
Δ <i>fpvA</i>	Deletion of <i>fpvA</i> gene generated previously in PAO1	Ochsner, et al. ³
Δ <i>pqsA</i>	Deletion of <i>pqsA</i> gene generated in PAO1	This Study
CF-102	Mild CF <i>P. aeruginosa</i> lung isolate	R. Ernst
CF-107	Mild CF <i>P. aeruginosa</i> lung isolate	R. Ernst
CF-135	Severe CF <i>P. aeruginosa</i> lung isolate	R. Ernst
CF-108	Severe CF <i>P. aeruginosa</i> lung isolate	R. Ernst
MRSA-M2	Methicillin-resistant isolate of <i>S. aureus</i> isolated from an osteomyelitis patient in Galveston, Texas.	Harro, et al. ⁵
<i>Plasmids</i>		
pΔ <i>pqsA-suc</i>	<i>pqsA</i> deletion suicide vector constructed previously	Farrow, et al. ⁶

Strain References

1. Taylor, R.K., Manoil, C. & Mekalanos, J.J. Broad-host-range vectors for delivery of TnphoA: use in genetic analysis of secreted virulence determinants of *Vibrio cholerae*. *J Bacteriol* **171**, 1870-8 (1989).
2. Holloway, B.W. Genetic recombination in *Pseudomonas aeruginosa*. *J Gen Microbiol* **13**, 572-581 (1955).
3. Ochsner, U.A., Wilderman, P.J., Vasil, A.I. & Vasil, M.L. GeneChip expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: identification of novel pyoverdine biosynthesis genes. *Mol Microbiol* **45**, 1277-87 (2002).
4. Banin, E., Vasil, M.L. & Greenberg, E.P. Iron and *Pseudomonas aeruginosa* biofilm formation. *Proc Natl Acad Sci U S A* **102**, 11076-81 (2005).
5. Harro, J.M. et al. Draft Genome Sequence of the Methicillin-Resistant *Staphylococcus aureus* Isolate MRSA-M2. *Genome Announc* **1**(2013).
6. Farrow, J.M., 3rd et al. PqsE functions independently of PqsR-*Pseudomonas* quinolone signal and enhances the *rhl* quorum-sensing system. *J Bacteriol* **190**, 7043-51 (2008).

Table S2. Real time PCR primers and probes used in this study.

Oligonucleotide	Sequence 5' – 3'
<i>Primers</i>	
<i>prrF.for</i>	AAC TGG TCG CGA GAT CAG C
<i>prrF.rev</i>	CCG TGA TTA GCC TGA TGA GGA G
<i>prrH.for</i>	ATT CGG CCG GAG ACG ACC GTT
<i>prrH.rev</i>	CGA CCA GTT GGT GTA ATA ATA ACT ATT
<i>antA.for</i>	CGC CAC CCT CGA CTA CAG
<i>antA.rev</i>	GGG CAT CTC GCT GAA GAG
<i>pqsA.for</i>	CCT CGA TTT CGA TCC CGA TAC
<i>pqsA.rev</i>	TGG CCT GGG AGA GAA TGT AGG
<i>pchE.for</i>	CGG CGA TCA ATA CCA TCG AC
<i>pchE.rev</i>	AAG ACC GAC AGA TCG AAG TCC A
<i>pvdS</i> for	CCT GGT CAA CTT CAT GAT CCG
<i>pvdS</i> rev	AGA TGG GTG ACG TTG TCG C
<i>Probes</i>	
<i>prrF</i>	TAA GCT GAG AGA CCC ACG CAG TCG G
<i>prrH</i>	CTG GCG ATG GAA TGA ATG AGA ACC G
<i>antA</i>	TCT CCT TCG CCA ACG GCC AC
<i>pqsA</i>	CAC TAT CGG GGC CAG ACT CTC AGC C
<i>pchE</i>	TGA ACG CAT CGG ATC GCT TGC TG
<i>pvdS</i>	CCT GGT GCA CTG CCG CAA GGT